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Anti-Inflammatory and Antipyretic Activities of Faloak (*Sterculia quadrifida* R.Br.) Stem Bark in Mice

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ABSTRACT

Faloak bark (*Sterculia quadrifida*, SQ) has traditionally been used in East Nusa Tenggara, Indonesia, for the treatment of various diseases. Previous studies have identified several bioactive secondary metabolites, including alkaloids, flavonoids, saponins, steroids, and terpenoids. This study aimed to evaluate the anti-inflammatory and antipyretic activities of SQ bark in vivo. Male Swiss Webster mice were used after acclimatization. Two preparations were evaluated: ethanol extract of Sterculia quadrifida (EESQ) for anti-inflammatory testing and ethyl acetate fraction of Sterculia quadrifida (FESQ) for antipyretic testing. EESQ was administered at doses of 100, 200, and 400 mg/kg BW, while FESQ was administered at doses of 50, 100, and 200 mg/kg BW. Anti-inflammatory activity was evaluated using carrageenan-induced inflammation, whereas antipyretic activity was assessed using a DPT-HB vaccine-induced fever model. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and saponins. EESQ demonstrated dose-dependent anti-inflammatory activity, while FESQ exhibited antipyretic effects. Higher doses showed stronger pharmacological activity in both models. These findings support the traditional use of SQ bark and indicate its potential as a source of bioactive compounds for managing inflammatory and febrile conditions. Further studies are required to investigate its mechanisms of action, long-term safety, and potential therapeutic applications.

Keywords: Sterculia quadrifida, Faloak, anti-inflammatory, antipyretic, traditional medicine

Introduction

Sterculia quadrifida (SQ) is a medicinal plant widely known by the people of East Nusa Tenggara (NTT) as Faloak or Flolo (Wewo et al., 2022). Empirically, SQ bark has been used in traditional medicine to treat various conditions, such as liver and kidney disorders, back pain, rheumatism, and digestive tract issues. Additionally, this bark is used to promote menstrual flow, cleanse postnatal blood, and enhance stamina (Siswadi et al., 2016). Previous studies have shown that the water extract of SQ bark contains water-soluble epicatechin flavonoids (Dean et al., 2019); Epicatechin is known to have various pharmacological activities, including analgesic,

antipyretic effects (Al-Sayed & Abdel-Daim, 2018) and anti-inflammatory effects. The anti-inflammatory activity of epicatechin is linked to its ability to inhibit the biosynthesis of eicosanoids, such as prostaglandins, which are key mediators in immunological responses. Prostaglandins are produced as end products of the cyclooxygenase enzyme, which plays a crucial role in various inflammatory processes (Robert J Nijveldt et al., 2020). This study demonstrates that flavonoids have significant potential as active compounds for supporting anti-inflammatory and antipyretic treatments, particularly for conditions associated with increased prostaglandin activity.

Fever and inflammation are common symptoms that often occur in various diseases, including liver disorders (Kementerian Kesehatan RI, 2018). The widespread use of these drugs in society, if not managed wisely, can increase the burden on liver function, particularly when combined with regular alcohol consumption. In NTT, the cultural practice of consuming alcoholic beverages, such as *tuak* or *sopi*, as part of traditional customs can further heighten the risk of liver damage (Boby et al., 2021; Hyun et al., 2021). Cloudy (24.8%) and clear (49%) traditional drinks account for the most significant proportion of alcohol consumption, with a combined total of 73.8%. This indicates that traditional drinks are deeply embedded in the culture of alcohol consumption in NTT (Kementerian Kesehatan RI, 2018), so special attention to liver health is critical. The use of SQ as a traditional medicine is expected to reduce dependence on conventional antipyretic and anti-inflammatory drugs, which have the potential to aggravate liver function.

The potential antipyretic effect of faloak bark extract has been proven preclinically, as research shows that this extract contains bioactive compounds such as flavonoids, saponins, steroids, and terpenoids, which exhibit antipyretic effects in vivo tests using Swiss Webster mice (Setyarini & Stefany Fernandez, 2016). The evidence of the anti-inflammatory and antipyretic effects of SQ extract offers a safer alternative treatment, especially to avoid the side effects of long-term drug use, such as cardiovascular disease, asthma, kidney injury, and the risk of exposure in pregnancy (McCrae et al., 2018). Traditional medicine has many advantages in the development of new drugs. Traditional medicine has been widely used in clinical practice and has diverse chemical structures and biological activities. This makes traditional medicine potentially capable of producing new drugs (Yuan et al., 2016).

Research on SQ bark remains limited and is not yet fully supported by strong scientific evidence. However, acute toxicity test results indicate that SQ bark does not cause toxicity symptoms or death in Wistar rats at doses up to 2000 mg/KgBw (Suci Noviyah A et al., 2021)and is safe at doses up to 5000 mg/KgBw in Sprague-Dawley rats (Siswadi dan Grace S. Saragih, 2018). These findings are crucial for detecting short-term toxic effects (BPOM, 2022) and provide a scientific basis for further developing its potential as a traditional medicine, particularly for fever and inflammation. Previous studies have mainly focused on identifying phytochemical constituents, evaluating toxicity profiles, and demonstrating the antipyretic activity of crude extracts of SQ bark. However, evidence regarding the anti-inflammatory

activity and the pharmacological evaluation of different SQ preparations, including ethanol extract and ethyl acetate fraction, remains limited and has not been comprehensively investigated in vaccine-induced animal models. This represents an important research gap in understanding the therapeutic potential of SQ bark.

Although SQ has been used empirically for generations with proven effects, evidence from preclinical and clinical trials is still needed. Preclinical trials strengthen the scientific basis for its use and provide clear guidance for developing faloak bark as a potential candidate for new drug discovery. Therefore, the novelty of this study lies in evaluating the anti-inflammatory and antipyretic activities of different preparations of SQ bark using a DPT-HB vaccine-induced mice model, providing additional scientific evidence to support its traditional use and future development as an evidence-based phytopharmaceutical candidate. The data from this study offer a solid foundation for selecting effective traditional medicines and minimizing the risk of side effects that could worsen a patient's condition.

Methodology

Materials

The plant material used for this study was the bark of SQ obtained from Liliba, Kupang, East Nusa Tenggara, and identified by the Jatinangor Herbarium Plant Taxonomy Laboratory, Department of Biology, FMIPA Padjadjaran University, with identification letter No. 31/HB/05/2021. The chemicals and reagents used include Pentabio (PT. Biofarma), 96% ethanol (PT. Bratachem, Indonesia), paracetamol (PCT) (PT. Kimia Farma), diclofenac sodium (DS) (PT. Kimia Farma), and 1% carrageenan (lambda)

Sample Preparation

SQ bark simplicia was air-dried for approximately one week to reduce the water content and then powdered to facilitate the extraction process. Extraction was performed using the maceration method with 96% ethanol as the polar solvent. A total of 1500 g of dried sample was macerated in 6.5 L of 96% ethanol for three days, followed by a remaceration process using 3.5 L of 96% ethanol to ensure optimal extraction of the active compounds. The maceration filtrate was then filtered and evaporated using a rotary evaporator at approximately 50°C until a thick extract was obtained. The resulting thick extract was further separated using a multistage fractionation method to obtain fractions with more defined components.

Characterization of the extract

The resulting extract was characterized by testing its water content and conducting phytochemical screening to identify the presence of alkaloids, saponins, tannins, flavonoids, and steroid/triterpenoid compounds.

Alkaloid Test

The extract was tested using Mayer, Dragendorff, and Wagner reagents. A positive test is indicated by forming a white precipitate with Mayer's reagent, a red-orange precipitate with Dragendorff's reagent, and a brown precipitate with Wagner's reagent.

Flavonoid Test

A positive result is indicated by the formation of a red, yellow, or orange color.

Saponin Test

The extract is considered to contain saponins if the foam formed remains stable for approximately 7 minutes.

Tannin Test

A positive result in the tannin test is indicated by the formation of a blackish-green or blackish-blue color (Harborne et al., 1996)

Animal Handling

The animals used in this study were male Swiss Webster white mice, aged 4-6 weeks and weighing 20-40 g. During acclimatization, the animals were kept under standard laboratory conditions with a temperature of 25°C-30°C and a 12/12-hour light/dark cycle. They were provided with standard food and boiled water. The test animals' welfare was strictly monitored per ethical guidelines throughout the experiment. Approval from the Ethics Committee of the Poltekkes Kemenkes Kupang was obtained. The minimum sample size was determined using Federer's formula, $(t-1)(n-1) \geq 15$, where t represents the number of treatment groups and n represents the number of animals per group. Based on the calculation, five mice were allocated to each treatment group ($n = 5$) to meet the minimum requirement for preclinical experimental studies while maintaining animal welfare considerations. Two preparations were evaluated in this study: Ethanol Extract of Sterculia quadrifida (EESQ) for anti-inflammatory testing and Ethyl Acetate Fraction of Sterculia quadrifida (FESQ) for antipyretic testing.

For anti-inflammatory activity, the mice were divided into five groups:

- G1a: Negative control (1% Na CMC)
- G2a: Positive control (Diclofenac sodium, 6.5 mg/kg BW)
- G3a: EESQ 100 mg/kg BW
- G4a: EESQ 200 mg/kg BW
- G5a: EESQ 400 mg/kg BW

For antipyretic activity, the mice were divided into five groups:

- G1b: Negative control (1% Na CMC)
- G2b: Positive control (Paracetamol, 65 mg/kg BW)
- G3b: FESQ 50 mg/kg BW
- G4b: FESQ 100 mg/kg BW
- G5b: FESQ 200 mg/kg BW

Anti-inflammatory Activity

The inflammation induction method involved injecting 0.1 mL of 1% carrageenan intraplantar into the soles of the mice's feet to induce inflammation. The anti-inflammatory activity of EESQ was assessed based on the diameter of the edema formed, measured using a caliper. Measurements were taken before and after

administration of the test preparation, with intervals of 10 minutes for 1 hour to monitor the changes that occurred.

Antipyretic Activity

The antipyretic activity of FESQ was evaluated by measuring the rectal temperature changes of mice every 30 minutes for 3 hours after administration of DPT-HB (diphtheria, pertussis, tetanus) and Hepatitis-B vaccines intramuscularly at a dose of 0.2 mL/20g BW. Mice with a temperature increase of at least 0.6°C after vaccination were selected to continue in this study. Both methods aim to evaluate the anti-inflammatory and antipyretic effects of SQ bark extracts and fractions and provide an overview of their therapeutic potential in overcoming these effects.

Statistical Test

The research data were analyzed statistically using IBM SPSS Statistics version 27 to determine whether there were significant differences between treatment groups. Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Data that met the assumptions of normality and homogeneity were analyzed using one-way analysis of variance (One-Way ANOVA) at a 95% confidence level ($p < 0.05$). When a significant difference was found among groups, the analysis was continued with the Least Significant Difference (LSD) post hoc test to determine specific group differences.

Result and Discussion

Sterculia quadrifida, known to contain flavonoids and alkaloids (Table1), shows the potential to overcome fever and inflammation. Several studies have shown that flavonoids have antipyretic effects by inhibiting the cyclooxygenase and 5-lipoxygenase pathways (Senthilvel et al., 2013); this inhibition reduces the release of arachidonic acid, which in turn inhibits the synthesis of prostaglandins, the primary mediators in the processes of fever, pain, and inflammation (Robert J Nijveldt et al., 2020). Additionally, in silico tests confirm that flavonoids (Masula et al., 2018)and alkaloids (Rachmania et al., 2018)can inhibit the cyclooxygenase enzyme, which is involved in forming prostaglandins as mediators of fever.

Extraction

The part of the plant used in this study was the bark of SQ, which grows on Timor Island, NTT. The simplicia was dried by airing, yielding 2400 g of dried simplicia from 5000 g of fresh bark (48% yield). Extraction was performed using 96% ethanol as the solvent with 1500 g of simplicia, producing 83.36 g of thick extract (5.5% yield). The thick extract was then fractionated using a multistage fractionation method, producing 2.48 g of thick ethyl acetate fraction (2.97% yield). Furthermore, a test to determine the water content in EESQ was conducted using a moisture balance. The test results showed that the water content in the faloak stem bark extract was 7.60%. This data is important for determining the moisture content in the extract, as it can affect its stability and quality before being used in further tests.

Table 1. Results of the identification of secondary metabolite content of EESQ

Compound group	Reagents	References	EESQ	FESQ
Alkaloid	Mayer	White to yellow precipitate	Positive	Positive
	Wagner	Brown precipitate	Positive	Positive
	Dragendroff	Red-Orange presipitate	Negative	Negative
Flavonoid	Mg Powder + Concentrated HCl	Color change from yellow to orange	Positive	Positive
Saponin	Hot aquadest, shake + HCl 2N	The foam is stable for 10 minutes and does not disappear after adding 2 N HCl.	Positive	Positive
Tanin	$FeCl_3$	Blackish-green color	Positive	Positive

Antipyretic Activity

Table 2. Temperature increase data after 30 minutes of DPT-Hb induction

Group	Average Temperature increase data (°C)	n
CMC	0.66 ± 0.17	5
PCT 65mg/Kg BW	0.84 ± 0.21	5
FESQ 50mg/Kg BW	0.76 ± 0.11	5
FESQ 100/Kg BW	0.72 ± 0.04	5
FESQ 200/Kg BW	0.78 ± 0.11	5
Average	0.75 ± 0.12	5

Administration of the DPT-HB vaccine effectively raised the body temperature of the test animals by more than 0.6°C within 30 minutes of induction (Table 2)(Kurokawa et al., 1974). Test animals experience increased body temperature above the normal temperature regulated by the hypothalamus as the body's temperature control center (Osilla & Sharma, 2019). This condition can be triggered by various factors, such as infection, inflammation, autoimmune disorders, use of certain drugs, or malignancy (Balli et al., 2023). The mechanism involves the release of immune mediators that affect the thermoregulatory center in the hypothalamus, causing an increase in normal body temperature.

Table 3. Changes in Body Temperature (°C) Following DPT-HB Induction at 30-Minute Intervals

Group	T0 (°C)	T30 (°C)	T60 (°C)	T90 (°C)	T120 (°C)	T150 (°C)	T180 (°C)
CMC	38.02	38.68	38.98*	39.06*	38.96*	38.72*	38.66*
PCT	37.94	38.78	38.44	38.32	38.22	38.10	38.02
FESQ 50	37.94	38.70	38.46	38.36	38.22	38.10	37.92
FESQ 100	38.04	38.76	38.58	38.44	38.36	38.20	38.06
FESQ 200	38.10	38.88	38.56	38.40	38.26	38.10	37.98

(* = $p < 0.05$ compared to all Test preparations).

Based on Table 3, Paracetamol, FESQ 50, 100, and 200 mg/Kg BW showed antipyretic effects in less than 30 minutes after preparation administration (*$p<0.05$). The data in Table 3 shows a decrease in temperature of 0.34°C in the paracetamol group, which is almost the same as the FESQ group, which has a 200mg/Kg BW dose of 0.32°C. However, this study found that the increase in dose was not comparable to the decrease in rectal temperature. The statistical analysis results did not show a significant difference between the positive control and test groups. The decrease in temperature is thought to be related to the content of one or a combination of secondary metabolites in SQ, which plays a role in inhibiting prostaglandin synthesis through its effect on the cyclooxygenase enzyme (Senthilvel et al., 2013). This mechanism provides the antipyretic effect of SQ, which has been proven preclinically.

In this study, body temperature decreased in the negative control group, which only received CMC. This decrease was attributed to the body's normal physiological response, where it attempts to normalize the temperature after the initial increase caused by the administration of the DPT-Hb vaccine as an inducer. This demonstrates the body's ability to regulate its temperature and return to normal conditions. Although the body temperature decreased, the negative control group did not show a temperature lower than the baseline or the temperatures observed in the other test groups.

Anti-inflammation Activity

The percentage of inflammation inhibition can also indicate anti-inflammatory activity. The higher the percentage of inflammation inhibition, the better the anti-inflammatory effect. Edema caused by carrageenan occurs through the mechanism of induction of COX-2 (Cyclooxygenase-2) expression, which plays an important role in the synthesis of prostaglandins, which are the primary mediators in inflammation, pain, and fever (El-Shitany & Eid, 2019). As a non-steroidal anti-inflammatory drug, diclofenac sodium showed the highest anti-inflammatory activity in this study. DS was not significantly different from EESQ at doses of 200 and 400 mg/kg BW, indicating comparable anti-inflammatory effects at higher doses of the extract.

This anti-inflammatory activity can be influenced by the content of secondary metabolites that can affect the activity of the cyclooxygenase enzyme in inflammatory conditions due to carrageenan induction. In addition to flavonoids and alkaloids, Tannins can also reduce the levels of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, by suppressing the release of these cytokines and reducing excessive inflammatory responses (Chen et al., 2022). The comparable effect between DS and EESQ at doses of 200 and 400 mg/kg BW suggests that SQ bark has potential as a source of anti-inflammatory agents due to its bioactive phytochemical constituents.

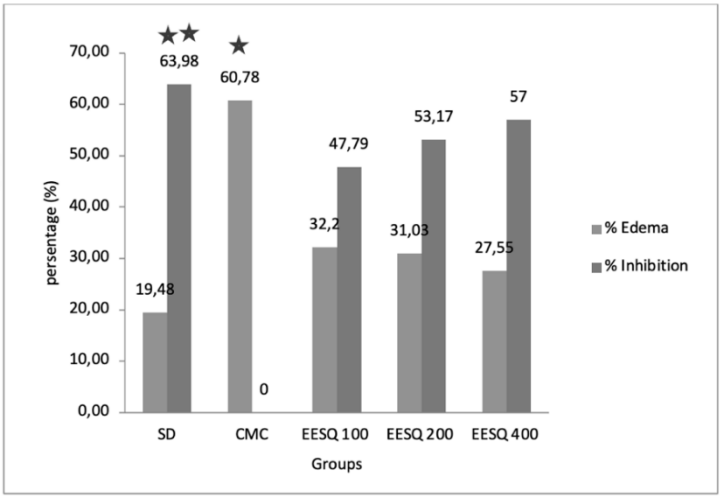

Figure 1. Anti-inflammatory activity of EESQ in carrageenan-induced mice model.

(* $p < 0.05$ compared to diclofenac sodium and all treatment groups; ** $p < 0.05$ compared to CMC and EESQ 100 mg/kg BW)

Figure 1 shows the anti-inflammatory activity of *Sterculia quadrifida* extract based on the percentage of edema inhibition in carrageenan-induced mice. A higher percentage of inhibition indicates stronger anti-inflammatory activity. Based on the analysis, diclofenac sodium (6.5 mg/kg BW) exhibited the highest inhibition (63.98%), followed by EESQ 400 mg/kg BW (57.00%), EESQ 200 mg/kg BW (53.17%), and EESQ 100 mg/kg BW (47.79%). These results indicate that all treatments demonstrated anti-inflammatory activity with a dose-dependent response in the EESQ group. Diclofenac sodium showed comparable effects to EESQ at doses of 400 and 200 mg/kg BW, while the lowest dose (100 mg/kg BW) produced a weaker anti-inflammatory effect compared to the higher doses and the positive control.

These findings suggest that SQ bark has potential anti-inflammatory properties that may be associated with its bioactive phytochemical constituents. Further studies using disease-relevant liver inflammation models, such as chemically or virally induced hepatitis, are recommended to better elucidate its therapeutic potential in inflammatory conditions.

Conclusion

SQ bark exhibited significant anti-inflammatory and antipyretic activities in vivo. The ethanol extract showed dose-dependent anti-inflammatory effects, while the ethyl acetate fraction demonstrated antipyretic activity. These findings support the traditional use of SQ bark and indicate its potential as a source of bioactive compounds for managing inflammatory and febrile conditions. Further studies are needed to explore its mechanisms of action and therapeutic potential in inflammation-related diseases, including liver disorders.

Declaration of Competing Interest

There is no conflict of interest in writing this scientific paper. All research results are compiled based on objective data and accountable experimental results.

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